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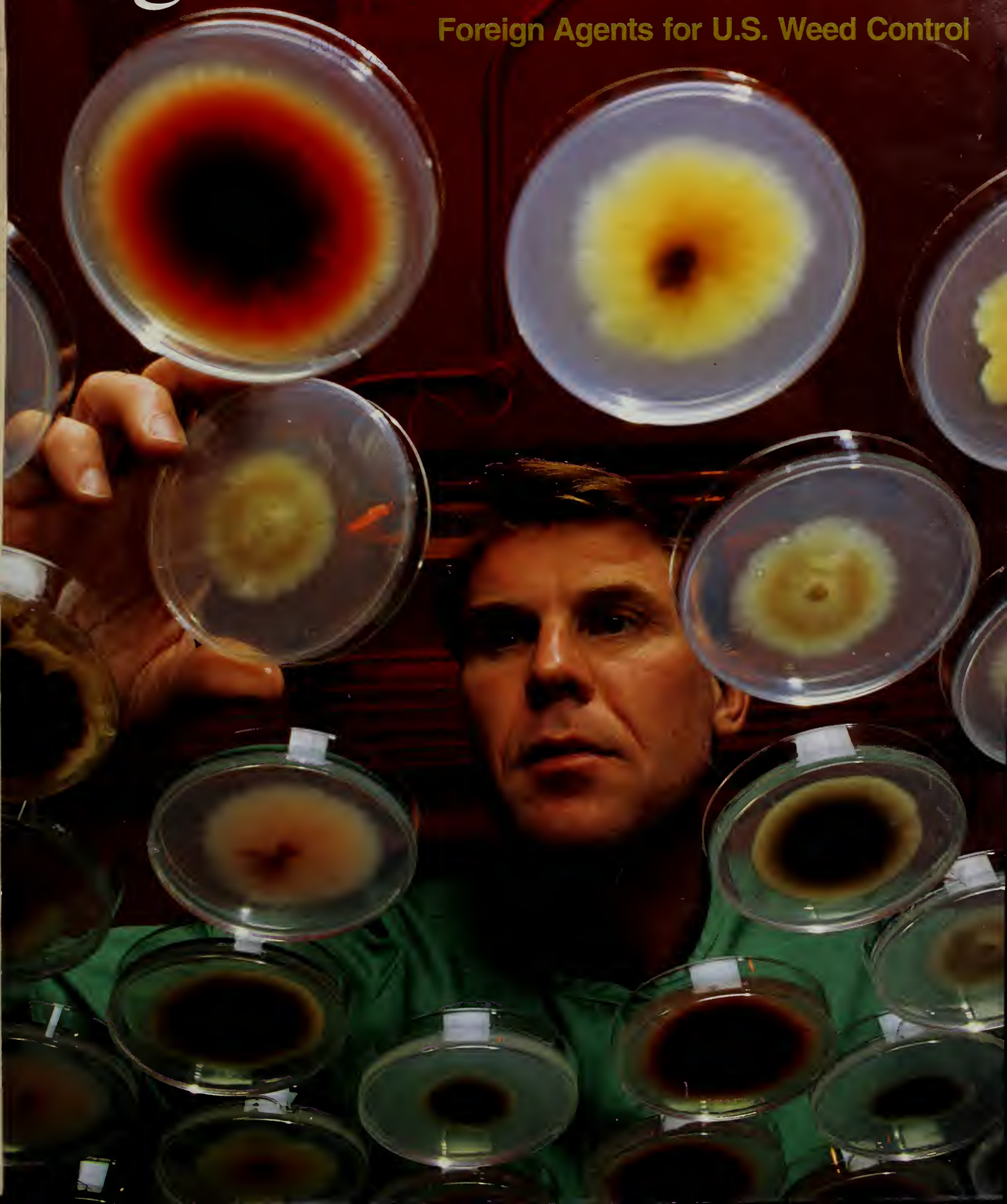
Department of Agriculture

Agricultural Research Service

May 1992

Agricultural Research

Foreign Agents for U.S. Weed Control



Michelangelo, Computer Security, and the Research Community

Not since the 16th century had the birthday of a Renaissance sculptor/painter aroused such an intensity of interest. Not to mention anxiety.

A computer virus named after the artist Michelangelo had infected personal computers from coast to coast. The virus was lethal—entirely capable of erasing a computer's hard-disk memory. Its spread was facilitated by users who share computer disks.

As the ominous date drew near, there was a flurry of activity in offices and laboratories throughout the nation. Floppy disks were feverishly backed up, diagnostic software was shoved into machine after machine, internal calendars were scrupulously reset.

Fortunately, the computing community had sounded an alarm, and most of us took protective action. Antivirus programs were used to identify contaminated computers and render their files clean. Backup copies of important files assured users that valued information bases would continue to exist. As it turned out, March 6, 1992, was not the computer Armageddon that fatalists had predicted; business—and science—went on.

Nevertheless, the incident had profound implications for all of us who depend on personal computers, and especially those of us who are employed in the research sector. In science, such betrayal by baud and byte is an essential risk; freely shared information is our bread and butter.

In ARS, virtually every scientist and technician has access to computers, and many use more than one system. In addition to mainframes and PC's, we depend on computer-driven laboratory equipment both for the sensing, collecting, and organizing of data and for its interpretation.

Consider, for example, field studies in which the effects of carbon dioxide on row crops are modeled. In these outdoor experimental rigs, computers control the flow of carbon dioxide into growth chambers, monitor and maintain amounts, record the responses of plants to the altered atmosphere, and then transmit the information to an indoor location where the data is backed up and manipulated.

If a virus were to interfere with any point on any link of these interdependent systems, an entire experiment's validity might be compromised. And since these experiments rely on the confluence of variables like weather and growing season, it might not be possible to replicate the experiment for some time—even years.

In terms of equipment, computerized instrumentation has revolutionized the ways we analyze substances, measure with precision, and examine even the tiniest of structures. The action we take may be driven by these precise readout capabilities. A virus released into a computer connected to a gas chromatograph at a soil analysis lab, for example, could lead to erroneous results in soil analysis and potentially to fields treated with inappropriate amounts of fertilizer.

Other sectors of agricultural research rely on large databases to manage information about pesticides, germplasm, and plant genetics—information that has been gleaned from many years of publicly funded science. Safeguarding such inestimable, irreplaceable banks of information is a matter of significant national importance.

Some government agencies, sensitive to the vulnerabilities of their computer systems, have instituted ironclad computer security policies designed to preclude information losses. Is such an across-the-board approach right for ARS?

R. Dennis Child, ARS national program leader for range and systems, has doubts. "I don't think we need an across-the-board policy for PC's in offices and labs so much as we need to educate ourselves about the problem. Once we are sensitized to the risk, individual units will as a matter of course take the measures they deem necessary."

At the same time, Child freely concedes, the potential for techno-vandalism suggests cautions that do have certain policy implications.

If you need to protect your work station from shared information, you need to be more cautious about letting someone else share your office equipment. And this makes it more difficult to participate in job-sharing. The practice of taking work home via diskette and continuing on your home computer may be risky, particularly if you share your home computer with others—perhaps a teenager who loves to exchange public-domain games and other software with friends.

In a sense, we have already paid the price of viruses like Michelangelo, in terms of what we have paid for up-to-date diagnostic software and in terms of the time we've had to set aside to examine our systems. And we've paid a price even if our files turned up scrupulously clean on March 6, 1992.

But imagine for a moment that they hadn't. How would we fix a price on the value of a lost computer file? In terms of the cost of its preparation? What about the demoralizing effect of having to rekey tedious but important charts that take weeks?

Most of us would agree with computer security expert Clifford Stoll, who inveighs against viruses because "they poison the communal well."

Regina Wigger
ARS Information Staff

Agricultural Research



Cover: Plant pathologist Rick Bennett examines fungi that may be used for biological control of pernicious weeds.
Photo by Scott Bauer. (K-4652-1)



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The Perils of Pulling...Weeds

One scientist's adventures in searching for thistles, spurge, and their diseases

He's been detained by security guards, stuck in food lines, stranded without gasoline, and chased by farm dogs—all while looking for plants that most people hope they never see.

Looking for weeds may sound like drudgery, but for Rick Bennett it has been an adventure amid the crumbling of the Soviet Union and Communist Eastern Europe.

Bennett, an Agricultural Research Service plant pathologist, now receives Christmas cards from his scientific collaborators in Russia—a sign of softening relations that took some of the thorns out of his search for weeds. But before that transformation occurred, Bennett found that searching for weeds was prickly business.

Since 1989, Bennett has spent about 18 months in more than a dozen European countries and the former Soviet Union, walking along roadsides in Romania, trudging through fields in Hungary, and sitting in the back of a Soviet military truck while being escorted through half a dozen former Soviet republics.

Bennett's looking for weeds with names like spurge and thistles, and for the disease-causing organisms that infect them. From numerous trips in 1989, 1990, and 1991, Bennett brought back more than 80 organisms that attack weeds in the United States. Several of these organisms are prime candidates as biological control agents—natural organisms that are environmentally safe alternatives to chemical herbicides.

Many of these weeds—such as leafy spurge, *Euphorbia esula* L., and yellow starthistle, *Centaurea solstitialis*—are thought to have originated in Europe and been brought here more than a century ago by immigrants who packed plants onto their ships when they set sail for America. In that soil, however, were weed seeds that would also find a new home in this country.

KEITH WELLER



Plant pathologist William Bruckart examines a fungus, *Puccinia carduorum*, on musk thistle in quarantined greenhouse. (K-4642-1)

The trouble is, the natural enemies of those weeds—such as insects and fungi—were left behind, or died on the voyage. So these imported weeds and others have swept across the United

KEITH WELLER



Leafy spurge stem infected with a fungus collected in Romania. (K-4645-13)

States, thriving without natural enemies. Since leafy spurge was first recorded in Massachusetts in 1827, it has spread across 2.5 million acres—mostly in the northern Great Plains—and costs ranchers an estimated \$35-\$45 million in damage each year. Yellow starthistle, since it was first recorded in California in 1869, now infests about 8 million acres in that state alone.

Without knowing it, the immigrants changed the natural balance of plants in the New World, giving these new weeds the upper hand. Helping to restore the natural order is Bennett's job. Among his tools are an aspirator, which he uses to collect fungal spores from weeds; petri dishes and test tubes in which he stores the organisms; and a portable microscope that he uses to identify them.

Bringing back weeds and their accompanying diseases is more complicated than it sounds. Because a fungus that Bennett finds in Romania doesn't already live in the United States, he can't simply bring it back and let it loose. He must get special permits to bring the organism back home, and it must be carried inside several sealed containers to make sure it won't escape if one of the containers breaks.

Those containers must then be transported into a high-tech quarantine lab at the agency's Foreign Disease-Weed Science Research facility in Frederick, Maryland. Bennett and colleagues must study the organism under strict quarantine regulations inside that lab, where air and water—even toilet and sink waste—are sterilized before they leave the lab. Anyone entering must change into lab clothes and shower and shampoo before leaving.

And the research payoff takes time. It can take up to 10 years to thoroughly study an organism and gain the necessary approval to release it in the United



In biological control studies, plant pathologist Rick Bennett collects fungal spores from yellow starthistle. (K-4645-1)

States. Much of that time is used to make sure other plants aren't affected by the foreign disease-causing organism. For example, there are 22 genera and 115 known species of the *Euphorbiaceae* family that are indigenous to the United States. (This family includes leafy spurge.) He must grow as many of those species as possible in the quarantine lab and expose them to whatever foreign organism he collects; if any of these plants shows signs of infection, the organism can't be used.

"We can only use organisms that are extremely specific to a certain plant species," Bennett says. "A lot of the organisms that I find can't be used in biocontrol because they attack plants other than the weed we're trying to control."

Aside from the scientific hurdles, bringing the organisms back to the

United States sometimes means convincing foreign police and security guards that you're really only on a scientific expedition.

That isn't easy. The nature of Bennett's work makes him seem suspicious to the eastern European peasants and police in the outlying villages where he spends most of his time. Most American tourists tend to visit European cities, taking in museums, cathedrals, and other urban attractions, but Bennett heads for the remote countryside where the weeds are.

"I'm always along roadsides or in fields, digging around, taking pictures, collecting, and isolating pathogens," he says. "My portable microscope almost always draws suspicion. In fact, customs agents in Romania took it apart once. They thought it was a camera containing microfilm."

Bennett often traveled by rental car in Europe, but not in the former Soviet Union. There, he was transported by the best available vehicle—a Soviet military truck, inside a canvas-covered compartment with only a tiny window and a buzzer. "If we were lucky enough to see any weeds out that window, we'd push the buzzer to signal the driver to stop. Then we would collect pathogens until it was time to return to camp, which consisted of several military-style tents."

There are few, if any, hotels and restaurants in the rural areas of the Soviet republics, and living and working conditions are harsh. On one visit to the former Soviet Union, he and his Russian scientific collaborators had to wait 2 days to simply get gasoline for the truck.

As an American traveling and working alone in Eastern Europe, Bennett sometimes faced additional problems—dealing with local police who were not accustomed to unfamiliar foreigners working in the countryside. An encounter in Czechoslovakia was typical. There, he and scientist Massimo Cristofaro, formerly an ARS entomologist based in Rome, were digging in a field of weeds when stopped by police. “We spent about 30 minutes trying to explain what we were doing—which was difficult because the police didn’t speak English and we didn’t speak Czech,” he says. Such confrontations were common in 1989.

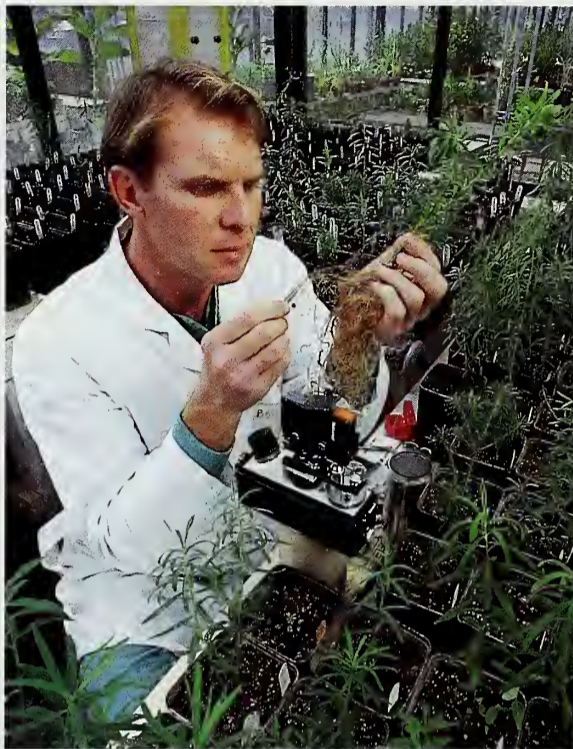
“A lot of times, I’d let them look through my microscope to let them see that there wasn’t microfilm in it,” Bennett says. “Luckily, the word scientist sounds the same in eastern European languages, so most of the time I could eventually get my point across.”

Making that point wasn’t always easy—especially in Romania, which, before the fall of the Ceausescu regime in December 1989, was one of the more hard-line Communist countries. Not only were security guards particularly suspicious, but food there was often scarce—as it was in many of the countries he visited. He had to stand in line for a handful of potatoes. A loaf of bread he bought was so hard he used it to prop up a table.

But Romania was one of the best places to look for weeds, because of its location near the Ukraine along the Black Sea—the area where leafy spurge is thought to have originated. It’s also a good place to search for weeds because of local farming practices; they don’t intensively plow their fields—meaning it’s a weed searcher’s dream come true.

But getting to the weeds was a challenge. In 1989, as a condition for conducting research in Romania before the revolution, Bennett and Cristofaro had to travel to predetermined places

KEITH WELLER



Rick Bennett examines roots of a leafy spurge from Russia for pathogens which could be used for biocontrol of this weed in the United States. (K-4641-1)

on approved roads. Their hotels—even the exact rooms—were arranged in advance. “If we deviated from the approved routes, we could be in for trouble.”

Even following the rules didn’t always help. While trying to leave Romania after a trip in 1989, he and Cristofaro were detained at the airport by security guards. Their passports were taken, their luggage was snatched from conveyor belts and searched, and they were interrogated for about an hour. Just when it seemed they might be detained for several days, a high-ranking official entered and let them go. There was only one flight leaving Romania each day, and they barely made it. “They told us never to come back.”

But they did—and to a markedly different reception. Ironically, during that next trip in the spring of 1990—after the fall of Ceausescu—the “same guards hugged us and said, ‘Welcome! We love Americans.’”

Despite all the obstacles, the trips were well worth the trouble from a scientific standpoint. In more than a

half-dozen countries, including Greece, Italy, Austria, Switzerland, Hungary, Romania, and Czechoslovakia, he found fungi, called *Uromyces*, that infect leafy spurge. One species, *U. scutellatus*, is a prime candidate for bringing that weed under control because, based on early lab studies, it appears to be specific to just leafy spurge. Scientists have known about the organism for several years, but progress has been slow. Bennett is now the only federal scientist working on the fungus.

U. scutellatus literally covered leafy spurge plants in Romania with black spores, called teliospores. The infected plants don’t flower and don’t produce seeds—effectively blocking reproduction, stunting growth, and deforming the plant stems. The fungus doesn’t infect seeds, which explains why it never stowed away aboard the immigrants’ ships with the weed seeds.

Since November 1990, Bennett has been studying *U. scutellatus* under quarantine at the Frederick lab. The main stumbling block so far is that it has been difficult to germinate the fungal spores in the quarantine greenhouse. Bennett has been trying to duplicate the fungus’ native climate, by determining the best conditions—temperature, moisture, light, and other factors—under which to germinate the spores.

He’s found that compounds produced by leafy spurge plant roots stimulate germination of the *U. scutellatus* teliospores. He’s also determined that optimal conditions for germination include 24 hours of continuous daylight, at a temperature between 20°C and 22°C.

Germination is also enhanced when the teliospores are exposed to some naturally occurring organic compounds, such as benzonitrile and beta ionone. These aromatic compounds are naturally produced by a wide variety of plants and are effective spore stimulators.

Bennett expects it may take up to 5 years to test other *Euphorbia* species related to leafy spurge, to make sure that they will not become infected by *U. scutellatus*. Then field studies will be necessary, so it could be a few more years before the organism is released to control leafy spurge in the United States. Until now, only insects have been released as biocontrol agents for leafy spurge.

Bennett and his colleagues are also studying other organisms for controlling other weeds. Several species of the *Puccinia* fungus are also under study for controlling yellow starthistle, purple starthistle (*Centaurea calcitrapa*), spotted knapweed (*C. maculosa*), diffuse knapweed (*C. diffusa*), and musk thistle (*C. thoermeri*).

Plant pathologist William Bruckart, also based at the Frederick lab, has been studying a musk thistle rust called *Puccinia carduorum* for several years and is awaiting approval from USDA's Animal and Plant Health Inspection Service to allow release of the organism in the United States. Such approval could come later in 1992 and would make *P. carduorum* only the second foreign disease organism ever intentionally released in the United States.

The first release of a foreign pathogen to control weeds in North America was in 1976, when the rust *P. chondrillina* was released to control skeletonweed, *Chondrilla juncea*, in California. That release was also made by the Frederick lab, in cooperation with the California Department of Food and Agriculture, and has been a major success in controlling skeletonweed.

Meanwhile, Bennett plans to continue his foreign travels. He and ARS range scientist Robert Masters of the agency's Wheat, Sorghum, and Forage Research Unit in Lincoln, Nebraska, are scheduled to take an exploration trip in May and June 1992 to collect leafy spurge in Russia, Ukraine, Romania, Hungary, and other

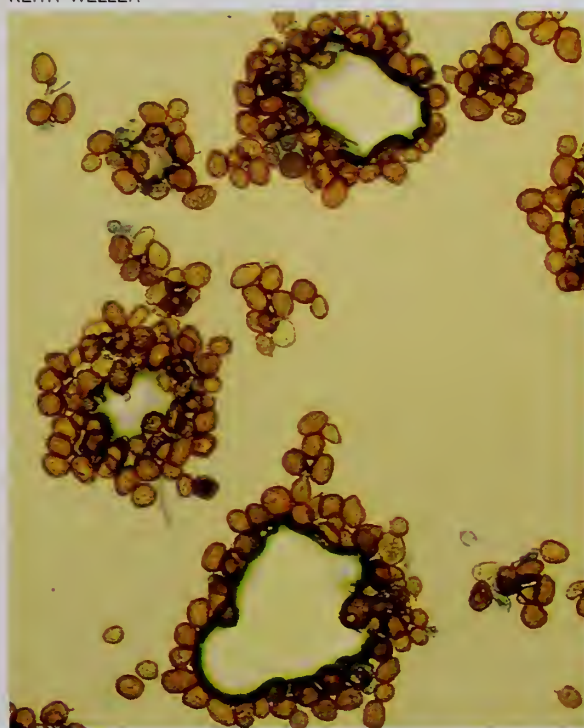
parts of Eastern Europe. The goal: To confirm the origin of leafy spurge and collect additional pathogens. Scientists have long suspected that area to be its origin, but they will verify that by bringing back leafy spurge varieties and comparing them to types collected across the United States. They'll use a genetic fingerprinting technique to make the matches.

"Once we determine the origin of leafy spurge, we can intensify our search for new pathogens. *U. scutellatus* is the best we've found so far, but there may be others. It's like putting together a puzzle. We still need more pieces."

Finding those pieces may become easier, given the recent changes in Eastern Europe and the former Soviet Union. But until he goes back, Bennett won't know whether his biggest challenge will be avoiding thistle thorns.—By **Sean Adams**, ARS.

Rick Bennett and William Bruckart are at the USDA-ARS Foreign Disease-Weed Science Research Laboratory, Fort Detrick, Bldg. 1301, Frederick, MD 21702. Phone (301) 619-7344. ♦

KEITH WELLER



Teliospores of *Uromyces scutellatus* enlarged about 30 times. Collected from leafy spurge in Europe, this fungus is a promising biocontrol for the same weed in this country. (K-4645-6)

Biocontrol Guidelines Stress Environmental Safety

Whenever ARS scientists study biological control organisms from outside the United States, they follow strict safety and research guidelines to ensure that insects, weed pathogens, and other organisms are thoroughly studied before they are released here.

Those guidelines have been published in a document called Biological Control Quarantine: Needs and Procedures. The 336-page document was issued in December 1991.

"It outlines standard procedures that natural enemies we import into the United States are safe to release," says Richard Soper, the ARS national program leader for biological control. He chaired a committee of scientists from ARS, other agencies, and universities that developed the guidelines.

The guidelines apply to what is known as classical biological control—importing foreign weed diseases or insect enemies to attack weeds or insect pests that have been unintentionally brought into the United States. On average, five agricultural pests invade the United States each year, and every 3 years one of these becomes a major problem, Soper estimates.

Imported biological control organisms must be studied in quarantine, isolated from the environment at large. Scientists must also conduct an array of tests—called host-range studies—to make sure that a biological control agent is specific to a certain weed or insect. For example, a disease that attacked a weed but also caused damage to endangered species or domestic U.S. crops would be ruled out, Soper says.

Although the guidelines are for ARS scientists, researchers in other organizations may use them as well. Soper says the Food and Agriculture Organization in Rome, Italy, and the North American Plant Protection Organization may also adopt the guidelines for their research.—**Sean Adams**, ARS.

Pitting Fungus Against Fungus

They're feuding in Georgia. No, it's not the Hatfields and McCoys, but two fungi that are closely related. And Agricultural Research Service scientists are using this rivalry as a promising food safety tool.

When fungi such as *Aspergillus parasiticus* and *A. flavus* infect peanuts, they can produce a natural toxin known as aflatoxin, which means financial losses to peanut growers.

Aflatoxin contamination costs the nation's peanut growers an estimated \$25 million annually, according to the Peanut Advisory Board in Atlanta.

season drought stress would be invaded predominantly by the competitive fungus, which doesn't produce aflatoxin.

"It's in those last days before harvest when peanuts under drought stress are most susceptible to aflatoxin contamination," Cole says.

The U.S. Food and Drug Administration requires that grain and finished products with 20 parts per billion or more of aflatoxin not be sold for human consumption or animal feed with certain exceptions. One part per billion is equivalent to less than 1 drop in 10,000 gallons.

Blankenship chose three strains as prospective aflatoxin prevention tools. But first, the three had to pass a few tests.

Researchers wanted to see if these strains could survive the hot temperatures and dry soils where their toxin-producing relatives thrive. And they needed to make sure the good strains always failed to produce toxin.

It was also important to know if these strains were rich producers of persistent-survival structures called sclerotia, which should enhance survival and competitiveness in the soil.

In a 3-year study that began in 1987, the three scientists saw dramatic reductions in aflatoxin contamination when beneficial fungi were applied to the soil during conditions that were ripe for aflatoxin formation.

Peanuts from treated soil contained 11, 1, and 40 parts per billion aflatoxin in crop years 1987, 1988, and 1989. Meanwhile, those from untreated soil during the same period had aflatoxin concentrations of 531, 96, and 241 ppb.

"Significantly, the greatest effect was seen in edible peanuts," Cole says. "Aflatoxin concentrations in edible, treated peanuts in 1988 were by far the lowest ever observed during 9 years of research using a specialized environmental test facility."

While the parent *A. parasiticus* strain doesn't make aflatoxin, it produces a compound known as O-methylsterigmatocystin (OMS) that is chemically related to aflatoxin. With this in mind, Cole and co-workers developed two altered forms, or mutants, of the parent fungus that don't produce OMS.

"It is important that any mutant used as a biocontrol not produce any related chemical that is toxic or carcinogenic," Cole says. "One mutant was discarded because it produced versicolorin A, which is a distant chemical relative of aflatoxin."

The scientists evaluated the performance of the parent strain and the

JACK DYKINGA



Before now, the only known way to control aflatoxin contamination in peanuts before harvest was to prevent drought stress. (K-4295-5)

The peanut industry's goal is to eliminate this potent natural toxin by the year 2000.

Scientists at ARS' National Peanut Research Laboratory in Dawson, Georgia, have discovered an *A. parasiticus* strain that does not produce aflatoxin. They have applied for a patent on this non-toxin-producing strain for use in controlling the harmful *A. parasiticus* strains in peanut fields.

The toxin-producing strains found naturally in soil would be replaced by the non-toxin-producing strain that is added to the soil, says Richard J. Cole, a co-inventor and research leader at the Dawson lab. Peanuts subjected to late-

Many states and export markets are considering stricter tolerance levels for aflatoxin, intensifying the peanut industry's eagerness to eliminate any chance of contamination.

"The only known methods for controlling preharvest aflatoxin contamination in peanuts are irrigation or early harvest," Cole says. "But irrigation is an expensive option not available to most peanut growers—and harvesting early reduces quality and yield."

Scientists at Dawson initially isolated the first non-toxin-producing fungus in 1980. Eventually, Cole and coinventors Joe W. Dorner and Paul D.

JACK DYKINGA



The three main U.S.-grown peanuts are Spanish (top), Virginia (center), and runner peanuts (bottom). (K-4297-18)

mutant as biocompetitive fungi. They found that both fungi were very effective in controlling aflatoxin contamination in edible peanuts.

In 3 years of testing, neither the parent nor mutant strains ever developed the ability to produce aflatoxin, thus making them appropriate candidates for controls, Cole says. The parent and mutant strains also demonstrated that they were highly competitive in the soil, compared with native *A. parasiticus* strains that produce aflatoxin.

But Cole and colleagues observed an interesting shift between the fungal population of the non-toxin-producing strains and natural strains in treated and untreated soils.

"When the tests were complete, the overall fungal population was no higher in soils treated with the beneficial strains than in untreated soil," Cole says. "This is an important ecological consideration: one would not want to drastically alter the normal levels of fungal populations."

He says research is under way to determine the most effective concentration and proper timing for the beneficial *A. parasiticus* strains to be applied to preharvest peanut soils. Also, scientists are working on various formulations and methods for peanut growers to apply the nonchemical aflatoxin control.

Although aflatoxin contamination will probably not be completely eliminated with this approach, Cole says, coupling it with other available techniques could go a long way toward ensuring an aflatoxin-free supply of edible peanuts.—By **Bruce Kinzel**, ARS.

Richard J. Cole, Joe W. Dorner, and Paul D. Blankenship are with the USDA-ARS National Peanut Research Laboratory, 1011 Forrester Drive, S.E., Dawson, GA 31742. Phone (912) 995-4441. ♦



Verticillium lecanii grows out of pellets used for delivering this fungus to the soil, where it will attack the soybean cyst nematode. (K-4302-10)

Micro-Pests Inflict Macro-Headaches

But fungi and pheromones can spell relief

A mutant fungus and some sexual confusion made life difficult for soybean cyst nematodes last summer.

It should happen again this year, in expanded tests by the Agricultural Research Service. The payoff, 5 to 10 years from now, could be new, safe alternatives to conventional pesticides for one of the soybean farmer's worst enemies.

Recent figures aren't available, but in 1981, scientists estimated that the soybean cyst nematode, *Heterodera glycines*, cost growers \$420 million a year in crop losses.

The nematodes—transparent, microscopic, eel-shaped worms—drain nutrients from soybean roots. One hundred grams of soil may contain hundreds of nematodes.

Controlling the pests with chemical nematicides can be expensive,

says plant pathologist Susan L.F. Meyer, who is with the ARS Nematology Laboratory in Beltsville, Maryland. And some nematicides, she adds, have been taken off the market because of concern that they may pollute groundwater.

Today's soybean farmers have two other options. "They can rotate soybeans with a crop, such as corn, that is not a host for the nematode," she says. "Or they can plant a resistant soybean variety. But in many cases, nematodes that aren't affected by the plant's resistance rebuild to damaging levels after several years."

The Nematology lab—the only one in ARS devoted exclusively to nematode research—is searching for longer lasting, more effective alternatives.

In early June, at sites in Delaware and Maryland, scientists will begin the second outdoor test of two potential alternatives.

One is the female nematode's pheromone, or sex attractant—and some related compounds. The other is a fungus genetically altered by Meyer in the lab.

Former ARS microbiologist Robin L. Huettel and coworkers isolated and identified the pheromone. The related compounds were devised by chemist Albert B. DeMilo at Beltsville's Insect Chemical Ecology Laboratory.

Huettel, DeMilo, and Meyer have filed patent applications on their discoveries. The research trials are being run under a cooperative research and development agreement with Crop Genetics International, a company in Hanover, Maryland.

Huettel, the Nematology lab's research leader since 1986, recently joined another USDA agency, the Animal and Plant Health Inspection Service (APHIS) in Hyattsville,

Maryland. There, she serves as chief operations officer for planning and designing APHIS programs for eradicating plant pests, including nematodes, diseases, and insects.

Huettel will continue to keep a close eye on the tests, though.

"If the fungus and pheromone prove themselves in large-scale tests," she says, "farmers could use them singly or in tandem. The best control might be to use both in conjunction with resistant crop varieties. That way, nematodes should take much longer to rebuild to damaging numbers."

Double-Whammy Ends an Underground Lifestyle

When immature nematodes leave their eggs, they wriggle through the soil in search of a young, tender soybean root. Using a needlelike mouthpart called a stylet, they penetrate the root and begin robbing nutrients from cells.

When a male nematode matures, he leaves the root to seek a mate, drawn by the female's pheromone. As the pheromone grows in potency, it "tells" him he's nearing a female. He then begins a dancelike rite—coiling and uncoiling—in an effort to mate.

But if—as in the tests last summer—the male senses pheromone or a similar compound in all directions, he dances alone or not at all. Instead, he wanders, unable to home in on a female amid all the chemical "noise."

In 1989, Huettel and former ARS chemist Howard Jaffee identified the pheromone as vanillic acid. Isolating and identifying the pheromone was painstaking work that took about 6 years. Each chemical analysis required extracts from 40,000 female nematodes.

Huettel says the female nematode appears to make the pheromone by digesting lignin, the main component of the walls of the root cells that make up her diet.

SCOTT BAUER



Plant pathologist Susan Meyer examines cultures of a mutant strain of the biocontrol fungus *Verticillium lecanii*. (K-4302-13)

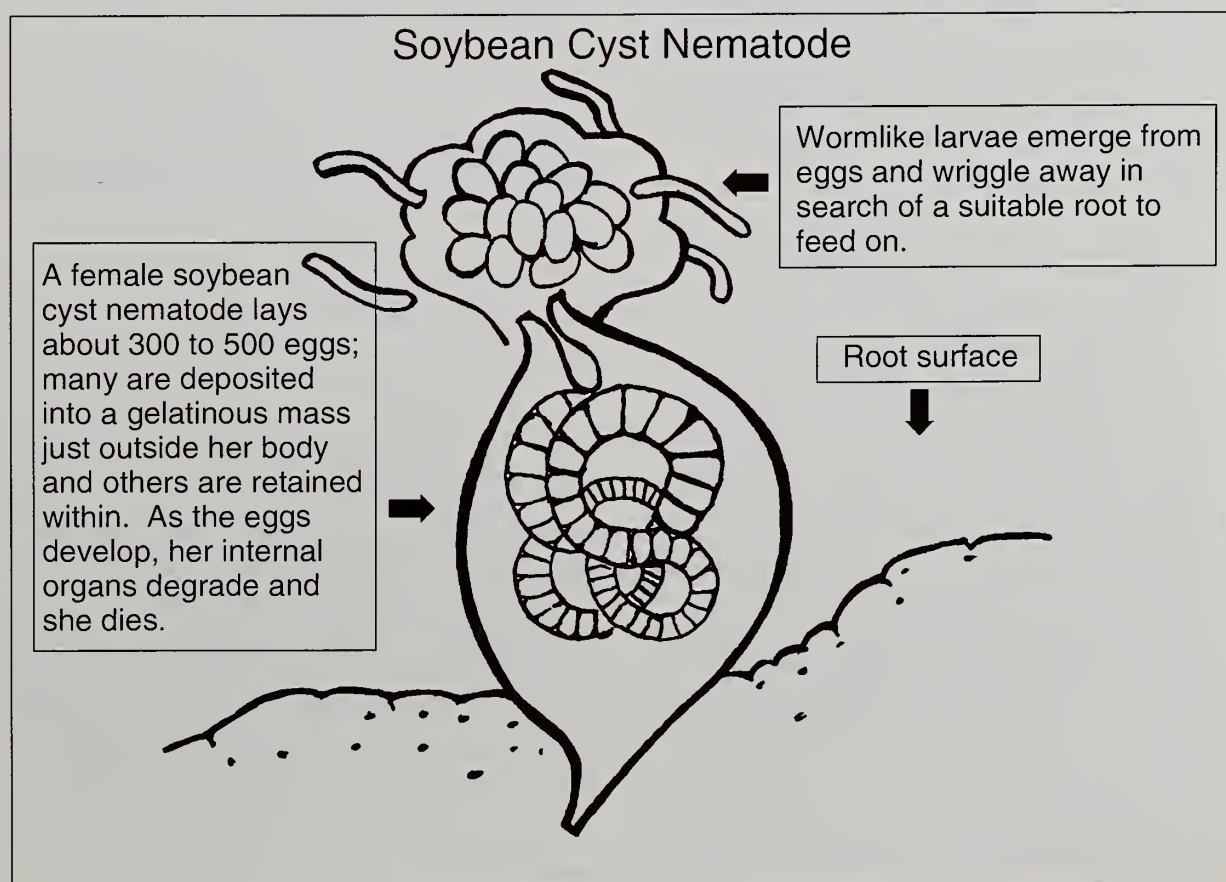
If the male nematode is fortunate and finds a female, he coils around her lemon-shaped body sac, deposits sperm, and soon dies.

The fertilized female lays about 300 to 500 eggs inside the sac and within a gelatinous mass just outside it. She dies shortly thereafter, and her body turns brown and only then is properly called a cyst. It serves as a protective incubator for the eggs inside it.

All this reproductive effort is for nothing, however, if *Verticillium lecanii* fungi prevent the eggs from hatching.

Meyer notes that this fungus had earlier been described as a parasite of the soybean cyst nematode. But the natural, or wild type, *V. lecanii* has a major weakness as a potential biological control agent. "The strains I tested were not very effective when applied at rates we consider practical for commercial uses," she says.

To try to develop more effective, hardier strains, Meyer designed a series of lab and greenhouse studies. She bombarded about 39,000 *V. lecanii*



spores with ultraviolet rays to induce potentially useful genetic mutations. She also exposed the spores to benomyl, a chemical registered for use on soybeans to control fungus diseases.

In earlier studies, she explains, scientists at Beltsville's Biocontrol of Plant Diseases Laboratory had found that some benomyl-resistant strains of other biocontrol fungi were more effective against their disease targets. "We wanted to see if that approach would work with *V. lecanii*. If so, we could improve its biocontrol potential while increasing its resistance to a fungicide that a farmer might be using."

Apparently, it did work. Meyer's studies yielded four promising strains of benomyl-resistant *V. lecanii* mutants. Meyer tested one strain outdoors for the first time last summer in Ingleside, Maryland. Some of the plots also had Huettel's nematode pheromone—or a similar compound.

Huettel ran additional, separate tests of the pheromone compounds in field plots in Delaware.

At Ingleside, nematode populations were 70 percent less on soybean plots protected by the fungus than on plots where nematicide was mixed into the soil. The pheromone did nearly as well, cutting populations by 66 percent.

"If the reductions hold up in further tests, either treatment would constitute an effective control," Huettel says.

When both the fungus and a compound related to the pheromone were used, reductions were 86 percent. "That's a dramatic reduction," she says, "but we need to repeat the tests on a larger area."

This test compound, syringic acid, was one of 29 compounds provided by DeMilo. Most have yet to be screened for activity against the soybean cyst nematode.

At the test site in Laurel, Delaware, soybean yields were about 30 percent

SCOTT BAUER



Looking for possible insect damage, technician Paula Crowley checks soybean plants at the ARS Nematology Laboratory. (K-4302-3)

higher on plots protected by syringic acid. Another compound increased yields 57 percent. Huettel cautions that repeated large-scale testing is essential to get a good idea of how the pheromone compounds or the fungi will benefit yield.

The fungus and pheromone are carried by alginate pellets originally formulated by scientists at the Biocontrol of Plant Diseases Laboratory in Beltsville and the ARS Southern Regional Research Center in New Orleans. The pellets consisted of a carrier made of pyrax, a finely ground material containing quartz, for the pheromone. Huettel and Meyer used a bran carrier for the fungus and fungus/pheromone combinations.

In the tests, pellets were placed atop each soybean seed at planting time and gradually released their active ingredients. This year, the researchers will place the pellets a centimeter or so away from the seed. "When we

determine the best placement, it will be one that farmers can easily do by adjusting their conventional planters," Huettel says.

For now, the scientists are focusing on making the fungus/pheromone duo work well on soybean cyst nematodes. But they say the approach might also work on other parasitic nematodes of field and orchard crops in the United States.

If so, farmers will have new, environmentally safe weapons against tiny worms that cause big problems.—
By **Jim De Quattro**, ARS.

Susan L.F. Meyer is with the USDA-ARS Nematology Laboratory, Room 152, Bldg. 011A. Phone (301) 504-5660. Albert B. DeMilo is with the USDA-ARS Insect Chemical Ecology Laboratory, Room 009, Bldg. 010. Phone (301) 504-6138. Both labs are at the Beltsville Agricultural Research Center, 10300 Baltimore Ave., Beltsville, MD 20705-2350. ♦

Woes of a Fruitless Diet

With the year-round availability of citrus fruits and juices, cantaloupes, strawberries, tomatoes, peppers, and leafy green vegetables, it's not hard to get the recommended daily intake of vitamin C. But some people still fall short, and there's now more evidence that it puts them at greater risk for high blood pressure, which can lead to heart disease and stroke.

In two epidemiological studies of relatively healthy Boston-area residents aged 60 to 100, researchers with ARS' Human Nutrition Research Center on Aging at Tufts and the New England Medical Center Hospitals found a strong relationship between elevated blood pressure and either low intakes or low blood levels of the vitamin. The difference was statistically independent of other variables that could affect blood pressure, such as age, sex, weight, smoking, dietary sodium-to-potassium ratio, and other dietary factors.

In one study, epidemiologist Paul F. Jacques with the ARS center used data collected from 410 volunteers, none of whom were taking medication for hypertension. Based on their own recorded food intake over a 3-day period, they were divided into five groups—ranging from those who took in less than 60 milligrams—the Recommended Dietary Allowance for vitamin C—to those who took in four times the RDA or more.

"The prevalence of high blood pressure was 1-1/2 to 2 times higher in people with vitamin C intakes below the RDA compared to all the other groups," says Jacques.

Compared to the group with the highest intake, those who got less than 60 mg/day averaged 11 millimeters of mercury (mm/Hg) higher for systolic pressure and 6 mm/Hg higher for diastolic pressure. That's an increase of 6 to 7 percent in both numbers.

But the advantages of vitamin C appear to have their limits. Jacques

In two epidemiological studies, researchers found a strong relationship between elevated blood pressure and either low intakes or low blood levels of vitamin C.

says his data suggest you cannot keep decreasing blood pressure by increasing vitamin C intake to well above recommended amounts. "We can't exclude the possibility that other nutrients in vitamin C-rich foods are keeping blood pressure in check," he says, noting that fruits and vegetables are rich in potassium, which is also known to counteract the blood-pressure-raising effects of sodium.

Although Jacques adjusted for sodium and potassium intake derived

from 3-day diet records, these records may not accurately reflect the volunteers' mineral intake. "It looks like a diet high in vitamin C is related to blood pressure but it may not be the vitamin C itself."

Jacques collaborated on a second study with Elaine S.K. Choi, an internist with the New England Medical Center Hospitals. Choi led a survey of 247 elderly Chinese Americans whose average plasma ascorbic acid (vitamin C) levels, dietary vitamin C intakes, and use of supplements were all lower than the elderly white population surveyed.

The researchers excluded volunteers taking medication for hypertension and divided the rest into four groups depending on their plasma ascorbic acid levels. The group with the lowest levels had blood pressure readings that averaged 21 mm/Hg higher for systolic pressure and 8 mm/Hg higher for diastolic pressure than the group with the highest ascorbic acid levels—differences of 16 and 9 percent respectively, says Choi.

Thirty-two percent of the participants had a history of hypertension, she says, and another 13 percent of undiagnosed hypertensives were found in the screening. Hypertension is of particular concern among Chinese populations, in whom stroke is the predominant cause of death from cardiovascular diseases.—By **Judy McBride**, ARS.

Paul F. Jacques is at the USDA-ARS Human Nutrition Research Center on Aging at Tufts, 711 Washington St., Boston, MA 02111. Phone (617) 556-3322. Elaine S.K. Choi, M.D., is with New England Medical Center Hospitals, Box 5, 750 Washington St., Boston, MA 02111. Phone (617) 956-6223. ♦



At the Subtropical Horticulture Research Station near Miami, horticulturist Raymond Schnell examines hybrid cocoa plants for resistance to witches' broom. (K-4635-3)

Breeding a Better Cocoa Plant for Chocolate Lovers

Smooth, rich, creamy, delicious. Melts in your mouth. Just a little chunk of it can give a quick burst of energy and relieve tensions and frustrations. Chocolate...according to a recent Gallup survey, it's Americans' favorite flavor.

But where does cocoa—the main ingredient in such deliciously decadent sweets as chocolate cream pie, mousse, and filled and coated candies—come from? Not from anywhere in the United States, but from *Theobroma cacao* trees that grow in Central and South America, Africa, the West Indies, Oceania, and Asia.

Raymond J. Schnell, ARS horticulturist at the U.S. Horticultural Research Laboratory in Miami, helps keep these trees healthy and productive. His research is an important link in the chain of events that brings raw products to U.S. chocolate manufacturers.

Through technology transfer, Schnell's research could help improve production and quality of cocoa worldwide. "Over the past 30 years, we've been trying to understand the genetics of *T. cacao*. But attempts to breed more productive and disease-resistant cacao haven't been very successful," he says.

"Witches' broom is one of the most ravaging diseases to hit cacao plants," he says. "We're working now to produce resistant trees."

In some northern areas of South America, losses from witches' broom can be almost 100 percent. This is the case in the region of Quevedo, Ecuador.

At the ARS Miami lab, Schnell tends about 200 cacao trees that he uses as parents of seedlings that he screens for resistance to witches' broom.

Schnell has traveled extensively in Central and South America, where cacao evolved, to bring back resis-

tant plants that might help against this disease.

"I visit countries where there are cacao trees that seem to escape this disease and bring back germplasm," Schnell explains. "We then cross-pollinate with trees growing here on the research station."

After a cross is made, it takes about 8 months to produce a seedling. From seedling to first harvest can take 5 years, with larger harvests requiring 5 to 8. So cacao breeding takes time. Given about 20 years, mature trees eventually grow to heights of up to 35 feet.

Schnell is looking for genetic markers that are linked to disease-resistance genes.

"In this analysis, we use protein and DNA from the plant in somewhat the same way that DNA from humans is used to detect predisposition to certain diseases," he says.

Catherine Ronning, a graduate student working with Schnell on cacao research, says, "If we find a particular enzyme pattern that appears to be correlated with disease resistance, then we can use this pattern as a genetic marker in screening for resistance to witches' broom. These markers will greatly reduce the amount of time required for disease screening."

"We have some very promising results from apparently resistant plants we brought back from a severely infested area of Ecuador in 1989," Schnell says. "We expect to release witches' broom-resistant stock to breeders within the next 2 or 3 years."

Witches' broom is caused by a fungus, *Crinipellis pernicioso*, according to Schnell's collaborator L.H. Purdy, who is with the University of Florida's Department of Plant Pathology.

"If a new branch of a cacao tree looks distorted with shoots overgrown, then you can be sure the fungus is at work," Purdy says. "The new growth configuration from an infected tree

KEITH WELLER



Biological laboratory technician Derek Harkins checks cocoa beans with white seed color that is linked to fine flavor. (K-4636-6)

L.H. PURDY



Green witches' broom on the end of a young cacao branch.

Cocoa Facts

Despite the similarity in names, the tree, *Theobroma cacao*, from which cocoa products come, is not related to the shrub that produces cocaine, the widely known anesthetic and drug.

Commercial cocoa, chocolate, and cocoa butter come from cacao seeds.

USDA's Foreign Agriculture Service (FAS) reports that the United States imported about \$1.1 billion worth of cocoa beans and cocoa products in 1990. Africa's Ivory Coast is the world's largest supplier, with Brazil the largest in the Americas.

"Raw cocoa products made up about 90 percent of this total," says FAS' Rex Dull. "But it also included imports of finished chocolate products for retail sale."

Retail sale of finished products made from these imports amounted to \$8 billion or more, according to the Chocolate Manufacturers Association.

The shrub *Erythroxylum coca* is the only known source of cocaine, which comes from the plant leaves. Compared to the 35-foot cacao tree, the coca is small, reaching only about 8 feet. Coca grows in Africa, South America, Southeast Asia, and Taiwan. Flowers on the coca shrub grow on short stalks instead of on the tree trunk like cacao.—By Doris Stanley, ARS.

looks like a bloated hand with swollen, distended fingers."

It's this strange-looking, fanlike structure resembling a broom that gives the disease its name. The broom, which contains the fungus, remains on the tree for a month or two, then turns brown, dies, and either stays where it developed or falls to the ground or lodges in the cacao tree.

"Wind carries the spores, and water activates the fungus," Purdy says. "Therefore, rainfall hitting these brooms (either on the tree or ground) stimulates production of small mushrooms that produce spores, which are the only infective part of the fungus. These spores reinfect other places on the tree or can float on the wind to infect other trees."

When they land on a wet tree, the spores germinate in about 2 hours and can penetrate plant tissue, causing infection in about 8 hours.

Cutting out the brooms from the trees would help prevent reinfection. So breeding trees to reach a more

KEITH WELLER



Horticulturist Raymond Schnell looks for parasitic witches' broom in cocoa pods that are ready for harvest. (K-4633-12)

manageable height—perhaps 12 to 15 feet—would be a significant help.

Cacao produces flowers differently from most other trees. Instead of

forming on branches like an apple or peach, buds grow directly on the tree trunk. Tiny cushions about the size of a quarter appear on the trunk. It's from these cushions that flowers and pods come. The pods remain on the plant for about 6 months; for the first 3, they are highly susceptible to witches' broom. If fungal infection occurs then, the cacao beans in the pods become unmarketable.

A chemical control is available, but the cost makes it impractical. Most cacao producers are harvesters, not farmers. They don't cultivate the trees, just harvest the beans. Income from the beans may be their only income.

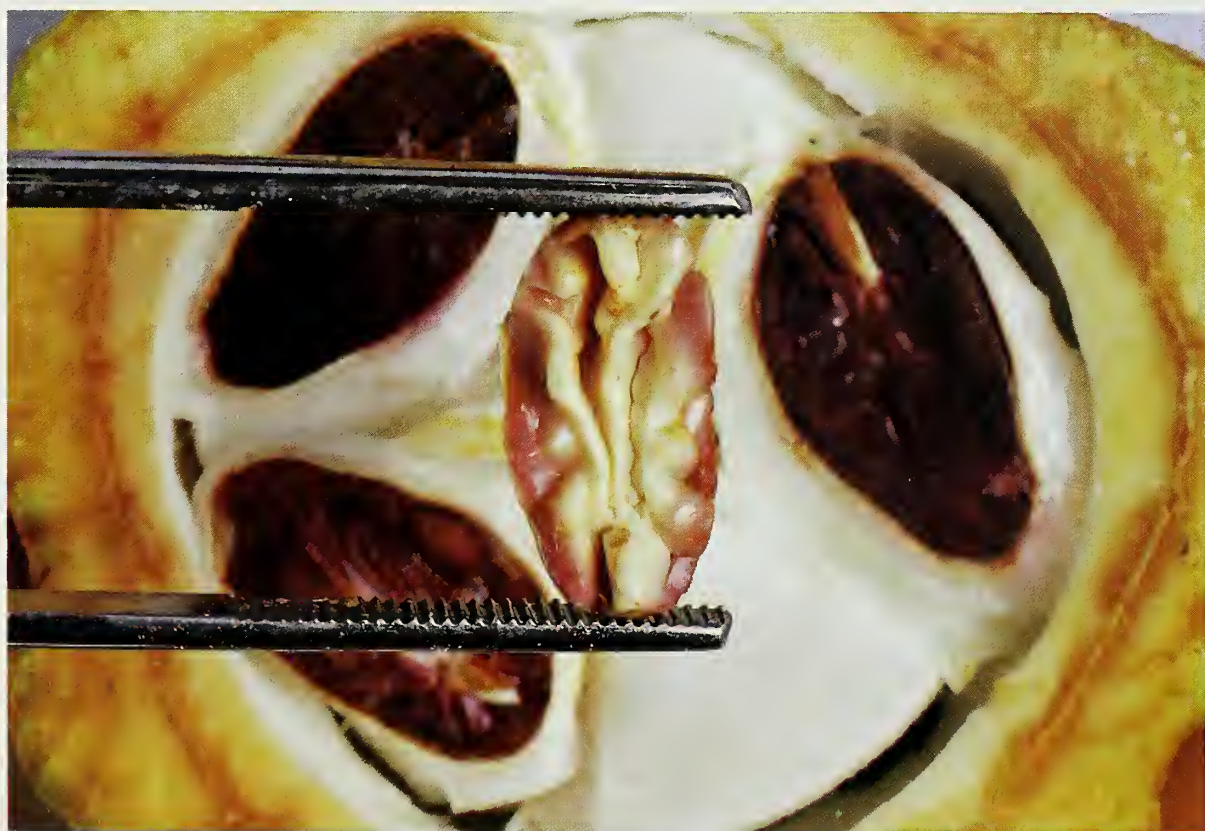
Also, the great height of the trees and hilly terrain in some countries make it difficult to spray, even if the harvesters could afford it. Then too, Purdy says, the chemical is a protectant. If the tree is already infected, spraying will neither stop the fungus nor cure the tree.

Although new systemic fungicides are available, the U.S. Environmental Protection Agency has not approved their use on cacao. Both Purdy and Schnell agree that finding resistant plants is the best answer. In addition to resisting witches' broom, they're also looking for a *T. cacao* variety that will yield well.

The American Cocoa Research Institute of the Chocolate Manufacturers Association and the U.S. Agency for International Development are collaborators on Schnell's research.—
By **Doris Stanley, ARS.**

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KEITH WELLER



Processors pay a premium for the lighter colored, more flavorful cocoa beans such as this one. (K-4637-3)

Lean on Me

Understanding age-related
loss of muscle tissue

It's no secret that our body composition changes as we age. Fat seems to pucker in all the wrong places while lean tissue—primarily muscle and bone—declines as we edge toward age 60 and disappears more rapidly afterwards.

The loss of lean tissue is much more than a cosmetic problem in spite of today's obsession with a youthful, muscular look. Based on studies of starvation and wasting diseases such as AIDS, "the maximum amount of lean tissue you can lose and still survive is 40 percent," says Ronenn Roubenoff, a rheumatologist and nutritionist with the ARS Human Nutrition Research Center on Aging at Tufts.

He and colleagues in the center's Body Composition Laboratory suspect that a person's lean body mass may be a good physiologic measure of age. Not chronological age, but rather of the changing ability to function in the world and to recover from illness. So researchers are looking into the question with additional funding from the National Institute of Diabetes, Digestive, and Kidney Diseases and from Pew Charitable Trusts.

Is the age-related loss of lean tissue programmed in the genes, or is it

subject to sedentary lifestyles or other correctable factors? "That's a major point of body composition research," says Roubenoff. "If we can figure out what governs lean body mass, maybe we can figure out how to maintain it."

To get a better handle on the process, Roubenoff, nuclear physicist Joseph Kehayias, and other colleagues

looked at the accelerated loss of lean tissue in people with rheumatoid arthritis—the most common autoimmune disease. "There's a very complex interplay between body composition and the immune system," says Roubenoff, noting that muscle loss can be stimulated by activating the immune system.

Thirty volunteers—20 with rheumatoid arthritis and 10 controls—were measured in a whole-body counter to get an estimate of their total body potassium. Since the element is found almost exclusively in lean tissue, it's a good yardstick of subjects' lean body mass.

Compared with the controls, the subjects with rheumatoid arthritis had 17 percent less lean body mass on average, says Roubenoff. "That's more than one-third of the lean tissue they can lose and still stay alive."

A major culprit behind this loss appears to be a substance secreted by certain immune system cells known as tumor necrosis factor, or TNF for short.

The researchers found that mononuclear cells from the blood of rheumatoid arthritics made seven times more TNF than normal. TNF was first shown to mobilize attacks against

SCOTT BAUER



Rheumatologist and nutritionist Ronenn Roubenoff uses a Tissot tank to collect and measure Trudy Hedrick's resting-energy expenditure. (K-4606-1)



Head nurse Leah Zanotti draws blood for analysis at the USDA Human Nutrition Research Center on Aging at Tufts University in Boston. (K-4607-3)

tumor cells, "but it attacks all cells," says Roubenoff. "Tumor cells are only slightly more sensitive."

Whether or not TNF also plays a role in the loss of lean tissue that comes with aging, "we don't know yet," he says. But if it does, we may be in luck. "Science is on the verge of blocking TNF."

Even without a blocker, TNF levels might prove to be a valuable biomarker for small losses of lean tissue in healthy people, indicating the need to increase exercise or improve nutrition.

The age-related loss of lean is often accompanied by a gain in fat. Roubenoff says, "The only healthy way to gain weight is to gain lean. But when we gain weight with age, we add about 20 percent lean and 80 percent fat."

He is one of many scientists calling for a better way to gauge obesity in individuals and in the U.S. population than body mass index (BMI)—which comes from dividing a person's weight by his or her height squared. Health policymakers rely on tables of average BMI's of healthy Americans that were developed by the Metropolitan Life Insurance Company. "Actually, we care about people's body fat rather than their weight," he says.

Trouble is, BMI doesn't give a true picture of how fat or lean people are, he says. Because of muscle weight, for instance, a football player would score high for BMI even with a very low-fat body. And many light-weight people actually have a high percentage of body fat. That's especially true in the elderly. The lack of more precise data has sparked a lively debate over whether or not it's okay to gain weight as we age, Roubenoff notes.

Another problem with BMI is that our height—the supposed constant in the ratio—doesn't remain constant. We tend to shrink as we age—primarily through compression of the vertebral column, says Roubenoff. But we don't all shrink at the same rate or to the

same degree. This can bias height-indexed measures of body composition, especially when comparing populations across decades of age.

The Shin Bone's Connected to the . . .

So he tested the potential of using the distance from the floor to the knee as a denominator that would remain constant. "Knee height accounts for one third of our total height," he says. He and Peter W.F. Wilson of the U.S. Public Health Service measured knee height and lean body mass in 610 people, aged 27 to 74, who are participating in the continuing heart study in Framingham, Massachusetts.

Although knee height decreased with age, he says, the fall was only one quarter that of total height in females and one fifth in males. When indexed

SCOTT BAUER



Nuclear physicist Joseph Kehayias positions Trudy Hedrick on the total body carbon scanner, which is used to measure total body fat. (K-4608-12)

to knee height, lean mass decreased in men as they aged, as expected. But this decrease did not show up when lean mass was indexed to total height, leading Roubenoff to suggest that knee-height is superior to total height as an index of stature.

The study used the relatively simple bio-impedance method, which estimates subjects' fat and lean mass by their resistance to a weak electrical current. The method is regularly used in research studies, and Roubenoff hopes it will become commonplace in physicians' offices over the next few years.—By **Judy McBride, ARS.**

Ronenn Roubenoff is at the USDA-ARS Human Nutrition Research Center on Aging at Tufts, 711 Washington Street, Boston, MA 02111. Phone (617) 556-3172. ♦

Carbon Rating Your Fat Status

Estimating body fat in elderly people calls for more straightforward measurements than the half dozen indirect methods currently used, says Joseph J. Kehayias of the Body Composition Laboratory at ARS' Human Nutrition Research Center on Aging at Tufts in Boston, Massachusetts.

Most of the methods commonly used in population studies estimate a person's lean tissue, or fat-free mass. Kehayias says small errors in this number can become magnified several times when it is subtracted from a person's total weight to determine fat mass. And the initial error may be further compounded in older people and those with underlying disease because the calculations are based on data from a younger, healthy population.

"We can't assume that older people are just like younger people," he says. For instance, differences in the location of their body fat or the elasticity of their skin may skew body fat estimates from skinfold measurements. "The lack of a reference for the elderly is a problem, he says. "We don't know which numbers are good."

So Kehayias, a nuclear physicist, has turned to measuring total body carbon as a more direct way to assess body fat. Nearly three quarters of the carbon in our bodies is in the fat, he says. Virtually all of the rest of it is in the protein, which can be measured quite accurately.

Sandia National Laboratory in Albuquerque, New Mexico, worked with Kehayias to develop a small generator that produces the fast neutron pulses needed for measuring body carbon. The generator exposes study volunteers to less than one quarter of the radiation they would get from a chest X-ray. Bursts of neutrons—the uncharged particles in atomic nuclei—raise carbon nuclei to the first-level excited state, he explains. They immediately drop back to the base state, giving off gamma rays, which are counted by detectors on either side of the body. "It's unique equipment and approaches the problem exactly the way we want to address it," Kehayias says.

He is more than halfway through a study of 220 subjects aged 20 to 100-plus, looking for changes in fat mass with aging. "It's the first time researchers have looked at aging without using a model developed for younger people," he says.

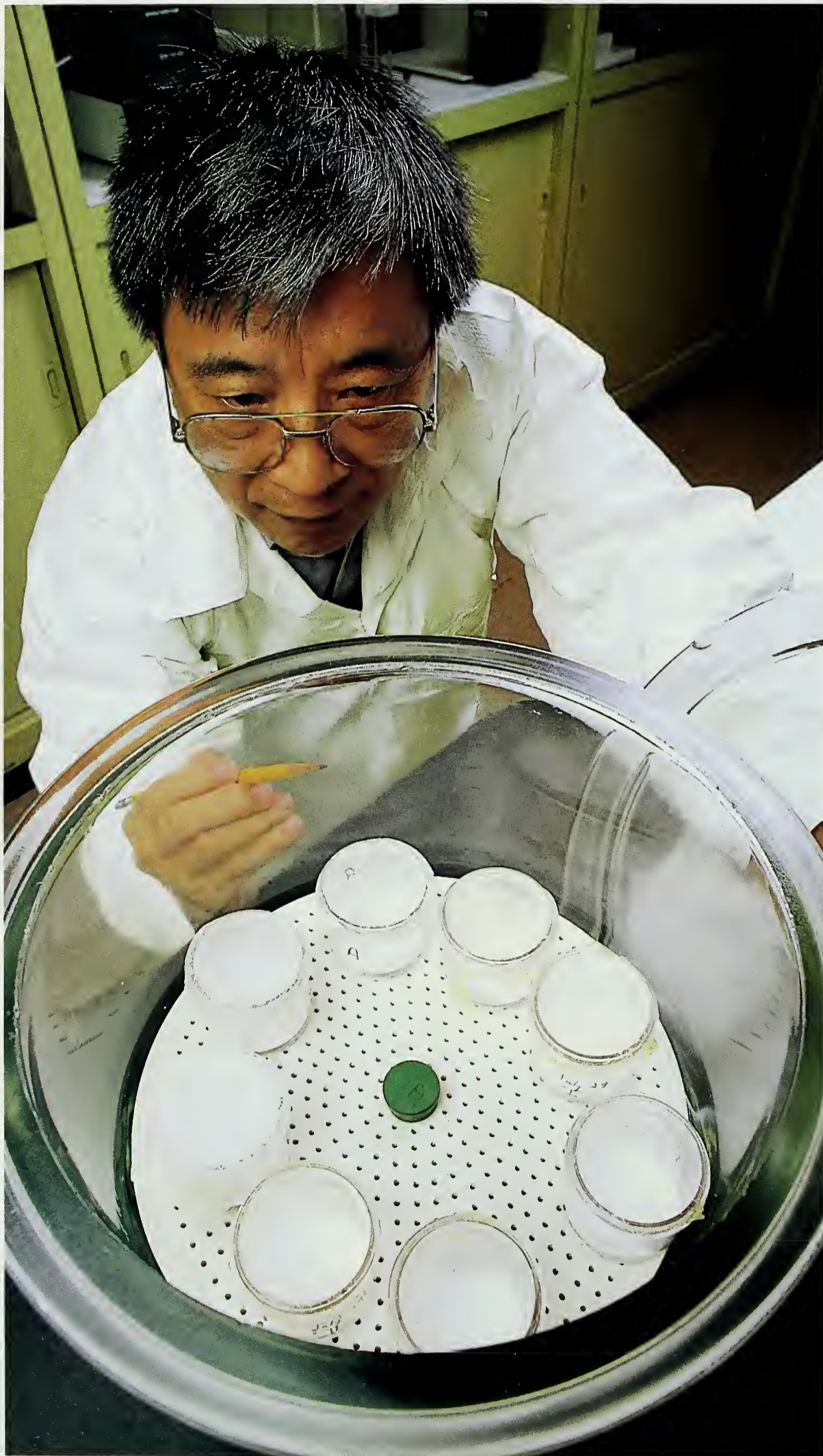
At the same time, he is estimating fat-mass by three of the indirect methods—skinfold, bio-impedance, and total body potassium—for comparison. "If there is a systematic difference, we'll find it," he says, so the indirect methods can be corrected for aging. But it may be that the methods will have to be revised from scratch with new data from the over-70 set.—By **Judy McBride, ARS.**

Joseph J. Kehayias is at the USDA-ARS Human Nutrition Research Center on Aging at Tufts, 711 Washington Street, Boston, MA 02111. Phone (617) 556-3162.

Protein-Rich Edible Coatings for Food

And biodegradable, as well, when used as packaging

SCOTT BAUER



Chemist Fred Shih analyzes the permeability of various soybean film types. (K-4472-8)

In the classic Frank Capra film “It’s a Wonderful Life,” the key character—George Bailey—is offered by an old school chum a “chance of a lifetime.”

That chance is a foot on the ground floor of a new business venture—soybean plastics. George Bailey—played by Jimmy Stewart—turns down the offer, and his pal winds up making a fortune in the business.

But chances like that only happen in the movies, right?

Try telling Frederick F. Shih of the Agricultural Research Service that such a notion is a pipe dream and he’ll answer that you’re wrong. A chemist at ARS’ Southern Regional Research Center in New Orleans, Shih’s found a new way to make a plasticlike material from soybeans.

“This research really has two benefits,” Shih says. “It provides a substitute for synthetic petroleum-based plastics and it promotes the use of surplus soybeans in the United States.”

Shih’s process differs from a 1940’s process for plastic auto bodies that simply incorporated soybean flour in phenol formaldehyde plastic.

Turning soybeans into films and coatings is a bit more complex than grinding the raw commodity, heating, and molding it into shape. Instead, Shih uses protein from the bean as the key ingredient.

In experiments at the New Orleans center’s Food and Feed Processing Research Unit, Shih first soaked soybeans and then ground them after their texture softened. Mixing ground soybeans with water separates the protein from the solid.

Shih freeze-dried the protein solution to remove water from the protein. The result is a fine protein powder. He is working with two types of soy protein powders. One, known as soy isolate, contains more than 90 percent protein. The second is soy concentrate, which has about 70 percent protein.

The proteins can be mixed with

various ingredients and additives before being cast into films or coatings for food products, Shih says. Sometimes, by using enzymes and other treatments, the protein can be modified for coatings and films with specific uses.

Other researchers have developed films and coatings from corn and wheat starches. But Shih says products made from soy protein may offer a bonus because of soy's adaptability for edible films.

"Protein can do a lot of things starch and gum can't do," says Shih. "It mixes better with oil, which is important in processing products and developing products with moisture-resistant characteristics."

And starch or gum can still be combined with soy protein to lend versatility to products. A common trait of films made of protein, starch, and gum is the ability to retain water and, at the same time, resist oxygen penetration.

By adjusting other factors, films and coatings can be tailored for effective food packaging and preservation.

For example, Shih says coating some food like meat products with these films generally retards moisture loss during short-term storage. But with time, the coating will eventually dry out and lose its ability to inhibit moisture loss from meat.

"Protein film actually attracts moisture, but its ability to repulse oxygen can also be significant for our purpose."

The soy protein coating could be useful in maintaining the original flavor of fat-containing foods. Precooked meat products, which might be used in institutional or school lunch programs, are particularly susceptible to developing an off-flavor, sometimes called warmed-over flavor.

"This defect is caused by oxygen penetrating the food and reacting with fats in the meat product," Shih says. "Coating food with protein films could solve the problem."

While moisture attraction and water migration may be a plus in some applications, they could be undesirable characteristics in other circumstances. In fact, an immediate goal of Shih's research is to reduce or control water permeability of the protein films.

Although edible coatings and films are essentially ready to use from a technical standpoint, they would first need approval from the Food and Drug

Administration. And Shih says it may take another 2 to 3 years of research before the agriplastic can be used for nonfood products like garbage bags.

"Realistically, it's very difficult to replace synthetic petroleum-based plastics because soy-based plastic would be expensive. But, if consumers demand an environmentally acceptable alternative to synthetic plastic, it could

SCOTT BAUER



The freshness of an apple slice is preserved by dipping it into a soybean protein solution. (K-4465-12)

SCOTT BAUER



Fred Shih inspects a protein film as it is peeled from its casting plate. (K-4463-6)

speed up research and development in this area that might lead to lower product costs."

Shih is currently studying ways to improve the strength, elasticity, and moisture resistance of plastics made from soy protein. He is also working with various additives that could help retard microbial growth.

"This is an alternative way to produce films, coatings, and other plasticlike products from a renewable resource," Shih says. "And, because they are made from protein, the products have the desirable feature of being edible, nutritionally valuable, and, most importantly, safe to the environment."—By **Bruce Kinzel, ARS.**

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Researchers Find New Use for Plumbers' Pipe

ARS veterinary medical officers Glynn H. Frank and Robert E. Briggs can be darned creative when it comes to checking cow tonsils for *Pasteurella haemolytica*, the bacterium that causes bovine pneumonia.

Research on bovine pneumonia has been going on since the National Animal Disease Center (NADC) opened in 1961. The disease costs the beef and dairy industry about \$800 million a year in medications, reduced growth rates, and livestock losses.

Frank and Briggs use polyvinyl chloride (PVC) pipe like a doctor's speculum to examine the tonsils of their cow patients.

"We needed lots of speculums so that we wouldn't cross-infect animals. And the PVC pipe versions were cheap and easy to make," says Frank.

They even put a small penlight flashlight inside the pipe to light the tonsils for the examination. "We also made a device to collect samples through the same pipe," says Frank.

From their field and laboratory studies, they hypothesized that healthy cows are often infected with *P. haemolytica*—but not in the nose or lungs, where the infection occurs during illness. While most researchers looking for the bacterium have searched in noses and lungs, Frank and Briggs have found it hiding in the tonsils.

The tonsils act as a reservoir for the *P. haemolytica* bacterium, along with an array of other bacteria. It can stay there, harmlessly, for over a month. Stress is a key factor in triggering the *P. haemolytica* to emerge from the tonsils and cause disease. The disease is worsened by a toxin, produced by the bacterium, that kills bacteria-fighting white blood cells in the lungs. While the bacterium is living in the tonsils, however, the cow produces antibodies that can neutralize the toxin.

Now, using the tonsil sampling technique developed by Frank and

Briggs, it will be possible to identify healthy carriers. That knowledge may enable researchers to treat *P. haemolytica* infections before they become a serious problem.—By **Linda Cooke, ARS.**

Glynn H. Frank and Robert E. Briggs are in the USDA-ARS Respiratory Disease Research Unit, National Animal Disease Center, P.O. Box 70, Ames, IA 50010. Phone (515) 239-8280. ♦

Blossom Thinner Ends Boom/Bust Cycle

An environmentally friendly chemical may soon help apple growers harvest an abundant crop every year, says an ARS scientist in Wenatchee, Washington.

A well-tended, mature apple tree can produce as many as 5,000 apples every fall, but that's no thanks to nature. Left alone, most apple trees yield a large crop one year and few fruit the next. To counteract that boom-or-bust tendency, orchardists thin their trees each spring. They do so by spraying chemicals that damage about half of the tree's blossoms.

"The trees have fewer, but larger apples," says Max W. Williams, an ARS plant physiologist at Wenatchee. "Thinning ensures that the tree will have enough reserves stored to produce blossoms for next year's crop."

A widely used chemical thinner, Elgetol, was recently taken off the market. Williams found, however, that a chemical already registered by the U.S. Environmental Protection Agency (EPA) for use on other crops can likely provide a safe and effective replacement.

Williams has already tested the product on acre-sized plots of Red

Delicious, Golden Delicious, Gala, and Fuji apples, with excellent results. Some tests were done in Washington, while other were carried out in Australia and New Zealand. During October of 1990 and 1991, Williams worked in Australia and New Zealand with their Departments of Science and Industrial Research (similar to USDA) and a private company, Agrisearch Services.

When sprayed on apple flowers at low concentrations, the chemical damages the blossom's pistil, or reproductive organ, so it can't be pollinated by bees. No pollination means fruit will not develop. Fruit remaining on the trees is from flowers pollinated and fertilized before the spray is applied, or from flowers unopened at the time of spraying. Tests have shown the material is not harmful to bees.

Within 24 hours after spraying, the compound breaks down into urea and calcium sulfate—two commonly used fertilizer ingredients, according to Dale W. Rush, director of product development at Sacramento-based Unocal, the company that makes the chemical.

"Apple trees use those breakdown products as nutrients," Williams points out. Another advantage is that once the chemical has dried on the trees, weather won't affect the results—unlike some other thinners. With Elgetol, for example, wet, cold weather after the treatment could increase the chemical's action, resulting in accidental over-thinning.

Williams says the compound will be tested this spring on about 100 acres of apple trees in Washington, Oregon, and Idaho. It will be the largest test on apples to date. EPA granted a special permit allowing growers to use limited amounts of the product this year; it will be marketed under the name Wilthin. The results should provide new data needed to okay the compound for widespread use in apple orchards.—By **Julie Corliss, ARS.**

Max W. Williams is at the USDA-ARS Tree Fruits Research Laboratory, 1104 North Western Ave., Wenatchee, WA 98801. Phone (509) 664-2280. ♦

Tapeworm Test Near

A genetically-engineered bacterium may be the key to a test for a cattle parasite that causes tapeworm infection in humans.

In recent laboratory tests, a protein from the bacterium has been used in a blood test to detect the presence of relatively small numbers of the cystlike parasite.

"This parasite, *Taenia saginata*, doesn't harm cattle where it resides in the muscle tissue, but does infect people who eat undercooked beef.

In cattle, the infection is called cysticercosis. It is common in South America, Africa, and parts of Europe and is inspected for at U.S. slaughterhouses. Lightly infected beef is frozen to kill the parasites while heavily parasitized carcasses are incinerated," says Marcia L. Rhoads, a chemist at ARS' Helminthic Diseases Laboratory in Beltsville, Maryland.

When, in the past, conventional laboratory techniques did not lead to a practical test for the parasite, scientists decided to employ genetic engineering methods to develop a new, more accurate serological test for the cystlike parasite.

"The gene that codes for the protein or antigen was derived from the parasite, cloned, and placed in the bacterium *Escherichia coli*, which can be laboratory-grown to make almost limitless quantities of the antigen," says molecular biologist Dante S. Zarlenga of the Biosystematic Parasitology Laboratory, also in Beltsville.

"Extracting the antigen from bacteria is the only practical method to obtain enough material for our tests. Although it is possible to obtain relatively small amounts of the natural antigen from infected animals, the labor and expense needed to perform this work make it an unacceptable approach.

Producing the antigen in culture allows us to more easily extract and purify the protein with much less variance from batch to batch," says Zarlenga.

"In limited tests of calf serum," the researchers say, "the antigen was nearly 100 percent accurate in detecting calves infected with the parasite. In one trial, animals infected with as few as 50 cysts were detected after 21 days. A practical, easy-to-run, automated test for use in slaughterhouses is at least 2 years away."

An accurate test for cysticercosis is necessary because current meat inspection techniques of heart and other tissue at slaughter can miss over 25 percent of infected carcasses.

Rhoads and Zarlenga have filed a patent application which covers the use of the antigen in tests for cysticercosis and similar parasites, says Ann Whitehead, ARS patent coordinator.—By **Vince Mazzola**, ARS.

Marcia L. Rhoads is at the USDA-ARS Helminthic Diseases Laboratory (301 504-8761) and Dante S.

Zarlenga is at the USDA-ARS Biosystematic Parasitology Laboratory (301 504-8754). Both laboratories are at the Beltsville Agricultural Research Center, 10300 Baltimore Ave., Beltsville, MD 20705-2350. ♦

Lower Water Pressure, Less Water Waste

Many Great Plains farmers have found a way to stop wasting precious water with their center-pivot sprinklers, those quarter-mile-long versions of lawn sprinklers. Each quarter-mile-long pipe rotates in a circle within a quarter section of land (about 130 acres). To air travelers, the irrigated areas show up as green circles on a tic-tac-toe board of farmland.

What air travelers are unlikely to see are the nozzles responsible for the green crops: They water the plants with a forceful spray into the air.

Farmers looking for a gentler and more efficient way to water their crops have begun lowering the pressure and angle of sprinklers or dropping spray heads to just above the crops.

The gentlest method is known as LEPA, for low-energy precision applicators. LEPA attachments spray water from about a foot above the ground, just about like pouring water from a garden watering can. They use a mere 6 pounds per square inch (psi) of water pressure.

An ARS study recently documented LEPA's efficiency advantage.

Arland D. Schneider, an ARS agricultural engineer in west Texas, led the study. It was done in an irrigated field that contained four 3-foot-square plots, each contained by buried steel boxes, that are constantly weighed by underground scales they are poised on. Called weighing lysimeters, the devices measure soil weight gain caused by the addition of water.

By measuring the changes, Schneider could tell how much water reached the soil in the plots and how much was lost to the air.

With nozzles that shoot water at up to 60 psi, he found that 15 to 20 percent of it never reached the plots. At 30 psi, with nozzles about 5 feet above ground, the loss was cut to 10 to 15 percent. But with LEPA, only 4 percent of the water was wasted.—By **Don Comis**, ARS.

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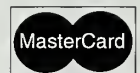
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